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Design, Implementation and Assessment of a Novel Intermittent Controllable Bioreactor for Supplying High Compression

Zhang Yu[1, 2, *], Li Pengsong[1], Sha Li[3], Cao Xuejiao[3], Dong Haichao[4], Song Kedong[2], Liu Tianqing[2]

[1]Department of Physical Education, Dalian University of Technology, Dalian, China

[2]State Key Laboratory of Fine Chemicals, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian, China

[3]Coll Basic Med, Dalian Medical University, Dalian, China

[4]Department of Orthopaedics, Second Affiliated Hospital of Dalian Medical University, Dalian, China

Email address

zhangyu0705@dlut.edu.cn (Zhang Yu)

[*]Corresponding author

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Abstract

One third of meniscus is white-white zone, composed of chondrocyte and extracellular matrix composition, and its regeneration ability is extremely limited. That leads to the damage which is not cured itself. At present, with the rapid progress of the construction of the meniscus in vitro, tissue engineering is the promising method for this question. Bioreactor systems play an important role in tissue engineering, as they enable reproducible and controlled changes in specific environmental factors. This work is based on afore research to design of a three-dimensional, hydrostatics multi parameter simulation, to simulate human menisci microenvironment system, meeting the scale expansion of seed cells, designing and constructing an intermittent controllable bioreactor with high-compression for reconstruction of meniscus in vitro.

Keywords

Intermittent, Controllable, Bioreactor, High-Compression, Meniscus, Three-Dimensional Construction

1. Introduction

The concept of tissue engineering in the 80's of last century which has become a new subject. The focus on research is the combination of cells and biological materials with biological activity, in vivo tissue or organs, in order to maintain, repair, regeneration, and improve the structure, function of tissues and organs of the loss. Therefore, tissue engineering combines biological science and engineering science. As for constructing engineered tissues and organs in vitro, physiological conditions of three-dimensional environment simulated in vivo is extremely important. Since 1960's, the method [2] is the most effective method, which is that Van Wezel cultivated animal adherent cells in microcarrier in vitro by microcarrier in a bioreactor. That method achieved large-scale expansion animal and plant cells. Generally speaking, the bioreactor is the application for tissue engineering, which also called reactor and the devices ought to meet the following basic requirements. The bioreactor of meniscus construction in vitro is a case study of bioreactor parameters, such as, bioreactor shape and function in order to facilitate the culture medium mixing, providing precise control of mass transfer, and maintaining a constant hydrostatic pressure or certain mechanical stress to simulate everyday life sports. In addition, the bioreactor needs combining with training for the perfect artificial matrix of cells and tissues. Recently, air lift bioreactor emerged, such as, hollow fiber bioreactor, single and double shaft rotating wall bioreactor, and spinner flask bioreactors.

Bioreactors can provide technical means to perform controlled studies aimed at understanding specific biological, chemical or physical effects. Furthermore, bioreactors allow for a safe and reproducible production of tissue constructs. For later clinical applications, the bioreactor system should be an advantageous method in terms of low contamination risk, ease of handling and scalability. To date the goals and expectations of bioreactor development have been fulfilled only to some extent, as bioreactor design in tissue engineering is very complex and still at an early stage of development.

2. Design and Engineering of the Bioreactor

The culturing parameters of tissue engineering bioreactor, in addition to cooperate with the features and needs of the aforementioned cell, meet the following basic requirements, such as, facilitating mixing the liquid culture, and providing precise control, making the nutrients, culturing liquid of pH gradient minimize, dynamic culturing cells within oxygen and nutrition matter passing more fully, and more easily excreting metabolites and cell phenotype more fully expressed. The mixed mode of culture fluid needs to be minimizing the damage of shear stress on cells. Cultivating a large number of stem cell differentiations, it is necessary to pay attention to the precise control of stem cells in different stages of differentiation required for different growth factors. Biological reaction device is designed to close to the in vivo situation development. Tissues and organs of human body are adaptation to different environment, such as meniscus with the stress of 6-10 MP in knee joint, thus that requires the reactor to provide a certain amount of mechanical stress, such as tendon in vivo mainly subjected to tension, and bone is mainly responsible for the compressive stress and cardiovascular mainly bear pulsating stress and other possible. Physical impact factor is still the magnetic field and the electric field. The design of a bioreactor must provide "specific" requirements for different tissues. Additionally, in order to meet the needs of large volume tissue construction on the number of seed cells, the combination of biological reactor and micro carrier cell culture technology is often being considered. Because the micro carrier has a larger specific surface area, as the same volume, larger than the average ones dozen times cells. Culturing cells reduces the phenomenon of cell differentiation, which is beneficial to the maintenance of cell phenotype and improve the quality of seed cells. Therefore, the aforementioned method maintains good combination of seed cells and micro carriers in the culturing process. Despite the above-stated requirements of tissue engineering reactor, recently, most of animal cell bioreactors reactors accomplish few improvements, though meeting the training needs of specificity. There are still many problems to be solved, thus bioreactors in tissue engineering field need great innovation and development. An integrated design methodology which incorporates theories, experiments and knowledge base has been formulated for the design of an anaerobic fluidized bed bioreactor. The design methodology starts with identifying the design objectives of the reactor and measuring the characteristics of the wastewater to be treated [3]. The capacity of tissue engineering meniscus to instead of the real is the function of the tolerance of mechanical pressure of the human body which tries to keep working itself. Bio-manufacturing factories of the future are transitioning from large, single-product facilities toward smaller, multi-product, flexible facilities. Flexible capacity allows companies to adapt to ever-changing pipeline and market demands [4]. To stimulate the internal environment is a breakthrough question.

2.1. Intermittent Controllable Pressure Bioreactor

With respect to tissue engineering, bioreactors are used for different purposes, such as cell proliferation on a small scale for individual patients or on a large scale for allogenic therapy concepts, generation of 3D tissue constructs from isolated and proliferated cells in vitro and direct organ support devices. These bioreactors enable controlling environmental conditions such as oxygen tension, pH, temperature, and shear stress and allow aseptic operation including feeding and sampling. Furthermore, a bioreactor system should allow for automated processing steps. This is essential not only for controlled, reproducible, statistically relevant basic studies but also for the future routine manufacturing of tissues for clinical application. Besides these global requirements, 3D tissue constructs based on cells and scaffolds are the specific key criteria, including the proliferation of cells, seeding of cells on macro-porous scaffolds, nutrient especially oxygen. The bioreactors supply the aforementioned materials and mechanical stimulation as a special device for meniscus reconstruction. Cell- proliferation is the first step in establishing tissue culturing. Usually, only a small number of cells can be obtained from a biopsy specimen. After expansion processing, several orders of magnitude are acquired. The cells proliferation is often accompanied by the dedifferentiation of cells. For example, proliferating chondrocytes show [5] a decreased expression level of collagen type II and an increased expression level of collagen type I. Small culture dishes such as petri dishes, 12-well plates, and T-flasks are generally used for cell expansion. These devices allow an increase in cell number approximately 10 times, thus other sub cultivations are emerged. These are considered as major devices for the dedifferentiation of cells.

Recent studies [6, 7] have shown that microcarrier cultures performed in well mixed bioreactor systems can significantly improve cell expansion. The cell seeding of scaffolds is an important step in establishing a 3D culture in a macroporous scaffold. Not only seeding at high cell densities, but also a homogeneous distribution of cells within the scaffold is essential. High initial cell densities have been associated with an enhanced tissue formation including cartilage matrix production, bone mineralization and cardiac tissue structure formation [8-10]. On the other hand, an inhomogeneous distribution of cells within the scaffold can significantly affect

the tissue properties. Critical issues of all bioreactor concepts involve mass transfer problems such as, oxygen and nutrient supply, and removal of toxic metabolites. The size of most engineered tissues is limited as they do not have their own blood system and the cells are only supplied by diffusion. Oxygen supply is particularly critical, [11, 12] as only cell layers of 100–200 μm can be supplied by diffusion. However, tissue constructs demand larger dimensions, and mass-transfer limitation is still one of the greatest engineering challenges. Some studies [13-16] showed that mechanical stimulation such as, mechanical compression, hydrodynamic pressure, and fluid flow, which are important modulators of cell physiology, have a positive impact on tissue formation, most of all, in the context of musculoskeletal tissue engineering, cartilage formation, and cardiovascular tissue. Though an abundance of evidence is that mechanical stimulation improves the properties of engineered tissues, only little is known about specific mechanical forces or the ranges of application such as, magnitude, frequency, continuous or intermittent, and duty cycle. Further studies of these factors coupled with quantitative and computational analyses of physical forces experienced by cells and changes in mass transport induced by the method are needed.

Bioreactors allow different process strategies including the batch, fed-batch or continuous cultivation. In particular, continuous perfusion enables cultivation under constant and controlled environmental conditions. Martin and his colleges [17] summarized some of the effects of direct perfusion on tissue-specific properties such as bone cells growth, differentiation and mineralized matrix deposition, human oral keratinocytes proliferation, albumin synthesis rates by hepatocytes, expression of cardiac-specific markers by cardio myocytes, and GAG synthesis accumulation by chondrocytes. On the other hand, a bioreactor system becomes more complex as additional features such as feeding pumps, vessels for fresh and spent medium, and control strategies, particularly in the case of mechanical stimulation. The bioreactor system has to be integrated into the entire cultivation scheme including biopsy, proliferation, cell seeding, tissue formation and delivery to the site of application. In many cases, the bioreactor itself is only used for tissue formation. However, for an approach from biopsy to the implantation of tissue, decreasing procedures should be considered, such as the number of steps, risk of contamination, and labor costs. This is particularly important with respect to the manufacture of engineered tissue constructs for clinical applications.

2.2. Determination of Major Design Parameters with Mechanical Force

The meniscus mechanical-biologic responses are the basic factor in therapeutic approaches to prevent degeneration and enhance repair of the meniscus. Studies [18] across a range of culture systems from isolated cells to tissues have revealed that the biological response of meniscal cells is directly influenced by physical factors, such as tension, compression, and hydrostatic pressure. In addition, these studies have provided new insights into the mechanotransduction mechanisms by which physical signals are converted into metabolic or pro/anti-inflammatory responses. Taken together, these in vivo and in vitro studies show that mechanical factors play an important role in the health, degeneration, and regeneration of the meniscus. Another culturing [23] way under dynamic conditions prior to implantation improved the neo-cartilage formation histologically and biochemically. The formation of cartilage was initiated by press-molding the mesenchymal bodies onto the surface of a bone substrate. Another study [19] tests it. By image-guided fabrication of the bone substrate and the molds, the osteochondral constructs were engineered in anatomically precise shapes and sizes. After 5 weeks of cultivation, the cartilage layer assumed physiologically stratified histomorphology, and contained lubricin at the surface, proteoglycans and type II collagen in the bulk phase, collagen type X at the interface with the bone substrate, and collagen type I within the bone phase.

Pore orientation mediated control of mechanical behavior of scaffolds and its application in cartilage-mimetic scaffold design. Further [20], these scaffolds demonstrated a highly viscoelastic behavior under cyclic compressive loading, with a pore orientation dependent relative energy dissipation. Comparing study [21] between the meniscus-like matrix and autologous tensioned synoviocyte neo-tissues (TSN) on histology, composition, and biomechanical properties are under consideration. Biomechanical properties [22] were determined by materials testing force–deformation curves. The evidences aforementioned concluded that mechanical stimulation are positive factor in the human mesenchymal stem cells (hMSC) differentiation and proliferation, and nanofiber scaffolds are potential for hMSC-mediated functional ligament tissue engineering.

3. Intermittent Controllable Bioreactor Designed by the Aforementioned Parameters

As the aforementioned parameters, we designed the intermittent controllable bioreactor (Fig. 1) for meniscus reconstruction in vitro. The controllable high pressure bioreactor mainly includes the main body of the biological reaction, the high pressure reaching 8~10MPa, and some parameters display components. The main body of the biological reaction is cylindrical, and the cavity body is provided with the amplification of biological cells and tissue engineering necessary construction. High pressure control system is used to control the reactor gas pressure and the parameter display module is used to display various parameters in the reactor. As in wherein intermittent controllable pressure biological reactor, bioreactor system training room inside and outside drum adopt withstanding 20MPa pressure stainless steel material. The intermittent controllable high pressure biological reaction system is characterized by the high pressure control system, including a quick interface, an air inlet valve, a back pressure valve and a

pressure meter. The quick interface is arranged in the reactor and connected with the gas inlet pipe. The air inlet valve is arranged at the top of the reactor stirring component and penetrates through the reactor to cause the gas to enter the reactor through a quick interface. The back pressure valve is arranged at the outside of the reactor and used to control the pressure value in the reactor. The pressure gauge is arranged in the reactor, through the reactor, and is used to display the gas pressure value in the reactor.

The intermittent controllable pressure biological reactor simulates human body environment to a great extent, which includes bone and cartilage tissue by pressure and three-dimensional culturing animal cells in vitro. Excellent culture system keeps genetic information and the inherent characteristics. Therefore, it not only reveals the basic mechanism of cell function in three-dimensional culturing environment, but also improves projecting cell quality, reduces products cost and accelerates the application of engineering organization. The inlet valve has regulation and control gas pressure in the reactor and the inside high pressure remains unchanged. Cell growth environment concludes 5% CO_2 concentration and 37 centigrade. Through the air inlet hose, reactor inlet is connected(interface with quick joints), opening the reactor inlet valve, back pressure regulating valve opening, inside the reactor filled with gas mixture, by adjusting back pressure valve opening to adjust the values inside the reactor gas pressure, gas mixture by the inlet pipe fed into the reactor and penetrates culture medium or after mini-pore filter directly through into fresh medium, and the pressure gauge displays the pressure value as a reference photo at any time control in the reactor pressure value. The inner and outer of the culture chamber of the rotary biological reaction system are driven by the adjustable speed motor through the gear drive, and the speed and direction of the electric control system be controlled at any time, reactor only the outer tube of the culture chamber is made of non-toxic, transparent and high temperature resistant poly carbonate material, and the rest are made of medical stainless steel material. An exhaust hole with a diameter of 2mm is arranged on the outer cylinder wall of the, and the flange of the outer cylinder is communicated with the training room, and the air exhausting hole on the outer wall of the outer cylinder is provided with a diameter of 180 2mm; inner cylinder in the middle not connected, in the not connected at both sides of, on the cylinder wall of the opening has a symmetrical distribution of 8 with a diameter of 1.5mm holes, the surface of the hole cover a layer of hydrophilic microfiltration membrane. The sealing system of reactor is made of silicone rubber 0 ring and bearing. A waste liquid outlet is orderly arranged between the discharging port of the reactor and the inlet of the peristalsis pump, and a liquid outlet is arranged in the order of three links and three links of a fresh medium inlet and an outlet. The medium oxygen filling device, the rotating bioreactor, the fresh culture medium tank, the waste liquid tank and the transmission medium and the gas pipeline are arranged in the heat insulation cover, and the temperature control in the heat insulation cover is controlled at 37 degrees centigrade by the electric control system, mixed gas inlet pipe and reactor inlet connected (interface with quick joints), reactor inlet valve is opened, by adjusting the back pressure valve opening to adjust reactor internal gas pressure, and through a pressure gauge to monitor the gas pressure value.

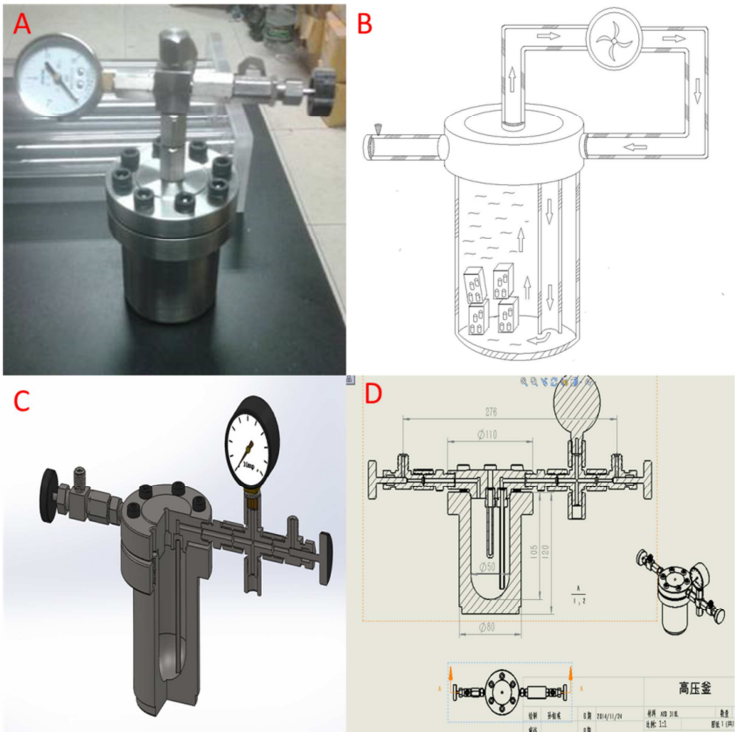

Fig. 1. *(a) Entity picture of the intermittent controllable bioreactor with high-compression. (b) Cultivating in intermittent controllable bioreactor with high-compression. (c) Design drawing of the intermittent controllable bioreactor with high-compression. (d) Schematic of the intermittent controllable bioreactor with high-compression.*

4. Bioreactor Cultivation and Findings

PC12 cells were used to test the bioreactor property. To extend PC12 cells in culture bottle, a bottle of cells as normal group, and another bottle of cells for pressure group. 16Mpa pressure was carried out on the PC12 cells for 5 minutes, once a day. The experiment is in progress for two days. Then, we respectively observe the microscopic cell morphological (Fig. 2) (Fig. 3) differences between two groups. Obviously, mechanical stimulated are positive to PC12 cells proliferation. From the graph (Fig. 2, Fig. 3), we found the cell morphology changes under the pressure of the cell in the reactor. Acceleration of value added rate is higher than that of control group.

5. Conclusions

The reactor can provide the culture of the cells under stress. Cell testing in a cell reactor is needed for further experimental research. A molecular biology level of testing, including the changes in the cell and inner proteins of the nucleus, is needed to be completed in further.

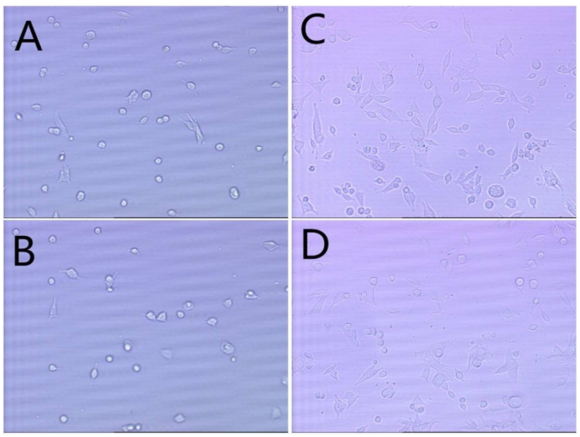

Fig. 2. *(a)(b) PC12 cells cellular morphology after culturing 4h, (c)(d) PC12 cells cellular morphology after 0.16 MP mechanical stimulate for 5 min, 2 times and then culturing 4h. (100x microscopic).*

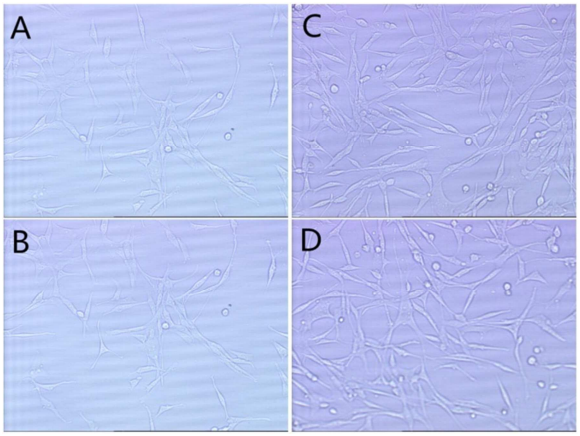

Fig. 3. *(a)(b) PC12 cells cellular morphology after culturing 24h, (c)(d) PC12 cells cellular morphology after 0.16 MP mechanical stimulate for 5 min, 2 times and then culturing 24h. (100x microscopic).*

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